Surface Modification of Hydrophobic Sphere with Dextran Generated from Enzymatic Reaction for Adsorption Site

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Abstract

Dextran was generated on the surface of the hydrophobic sphere via dextransucrase's reaction for the adsorption of concanavalin A. The hydrophobic sphere was polymerized in the range of 10 – 500 μm. The sphere was immersed in dextransucrase's solution and sucrose, a substrate for dextransucrase, was reacted with the immobilized dextranscurase. Due to the complexation of dextran with dextranscurase, dextran was produced on the surface of the sphere. The density of the immobilized dextransucrase on the sphere surface was changed from 8.8×10^{15} to 2.4×10^{16} number/m^2. The stability of the dextran-produced sphere in water was higher than that of the unmodified sphere because the produced dextran had the hydrate structure. The molecular weight of dextran produced was possibly calculated to be more than 10,000 kDa. Based on concanavalin A adsorption to the produced dextran, the appropriated density and length of the produced dextran were required for the smart adsorption of concanavalin A.

Keywords

Dextran; Dextransucrase; Adsorption; Con A; Enzyme; Modification

Introduction

The surface modification using saccharides has been studied for cosmetics, analytical materials, and separation materials. In saccharide engineering, saccharides were known as having an information transfer compounds between cells, applied for the recognition sites of virus and microorganism on the material surface (Hakomori, 2002). Saccharides have mainly hydroxyl group along with amino group, carboxylic group, and sulfonic group and polysaccharides are formed between monomer saccharides by glycoside bonding. To modify the material surface with saccharides, the chemical method is the promising tool (Piehler, et al., 1999; Xu & Logan, 2006; Barbucci, et al., 2006). The functional group is introduced to saccharides, attached to the active site of material surface. However, the molecular structure would be destroyed due to the chemical treatment of saccharides. For instance, hydrophobically-modified dextran was attached to the polystyrene sphere via hydrophobic interaction (Founier, et al., 1998). The reduced dextran was reacted with the amine group on the surface of silicon (Miksa, et al., 2006). During the emulsion polymerization, dextran was directly mixed with the monomer solution and then the polymerization was started to form the coating of the surface with dextran (Ladaviere, et al., 2007). Therefore, the sophisticated modification of material surface with saccharides in maintaining their molecular structure is under going in researches.

Dextransucrase (DSase) produces dextran from sucrose as a substrate. The produced dextran forms a complex with the active site of DSase (Mooser and Iwaoka, 1989). Dextran is polymer of glucose composing α-(1,6) glycoside bonding and has the random structure with high hydrophilicity due to the plural hydroxyl groups. Using the characteristic of DSase, the material surface is easily modified with dextran; DSase is immobilized on the material surface and then sucrose is reacted with the immobilized DSase, resulting the production of dextran on the surface. Until now, the dextran is generated on the pore of the porous membrane (Seto, et al., 2007; Kawakita, et al., 2007; Kawakita, et al., 2009b) and the surface of hydroxyapatite (Kawakita, et al., 2008). In the case of the membrane, DSase was immobilized on the membrane surface in a permeation mode and subsequently sucrose solution was flown through the DSase-immobilized membrane, producing the dextran on the membrane. The quantitative occupation by dextran in the membrane was determined by Kozeny-Carman equation (Kawakita, et al., 2009a). Also, dextran was generated on the surface of hydroxyapatite via DSase's reaction to control the adsorption of protein with the various sizes to hydroxyapatite (Kawakita, et al., 2008). Due to the steric hinderance of generate dextran, protein

adsorption was dependent on the size of protein. To the surface of the inorganic material, if DSase is possible to be immobilized as an active state, dextran is easily generated.

The surface of hydrophobic sphere doe not wet well with water. There are many papers regarding with enhancing the wettability with water on the surface of hydrophobic sphere (Cao, et al., 2009; Kaggawa, et al., 2006). For instance, the amino group or carboxylic group was introduced to the polystyrene sphere via chemical reaction. Saccharides have the high hydrophilicity. In introducing the saccharides to the surface, high wettability with water would be desired. Moreover, saccharides have been known as a recognition site of lectin molecules on cell surface. Through the recognition with cells between saccharides and lectin, the signal such as chemical transfers there. Researchers want to introduce saccharides easily and without destroying the structure of saccharides on hydrophobic sphere. However, direct introduction of saccharides on the material surface of hydrophobic sphere has the difficulty due to the less reactivity and regioselectivity of saccharides to the sphere via chemical reaction.

In this study, dextran was introduced to the surface of hydrophobic sphere via DSase's reaction for the adsorption of lectin. Nonporous hydrophobic sphere made from styrene-divinylbenzene was polymerized in suspension polymerization method. DSase was immobilized on the surface of the prepared hydrophobic sphere and then sucrose was reacted with DSase-immobilized-hydrophobic sphere to generate dextran on the surface, as shown in FIG. 1. Four kinds of dextran-generated sphere were prepared in changing the density and length of dextran for which the immobilization density of DSase and the reaction time of DSase immobilized with sucrose were controlled. Concanavalin A (Con A) has an affinity to glucose. The density and length of dextran on the sphere play the key role in the adsorption of lectin

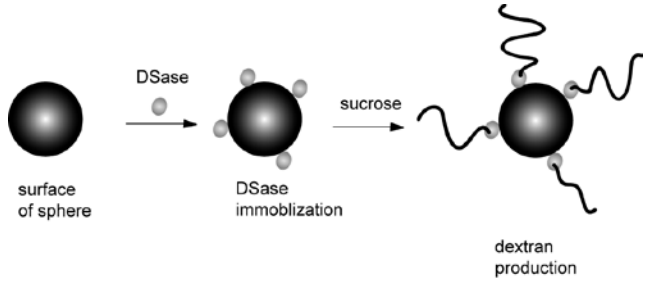

FIG. 1 ILLUSTRATED SCHEME OF DEXTRAN GENERATION ON THE HYDROPHOBIC SPHERE

(Chern, et al., 1998). The evaluation of Con A adsorption with dextran enables us to understand the state of dextran on the hydrophobic sphere.

Experimental Section

Materials

Dextransucrase (DSase) from *Leuconostoc mesenteroides* (D-9909 Lot No. 128H4026, 10 U-DSase/mg-DSase) was purchased from Sigma Chemical Co and used without further purification. One gram of powder is equal to 12.81×10^3 U. Styrene and divinyl benzene were purchased from Tokyo Chemical Industry Co. and Sigma-Aldrich Co., respectively. Polyvinyl alcohol (molecular weight 13-23 kDa) and benzoyl peroxide were obtained from Sigma-Aldrich Co. Other reagents were of analytical grade or higher.

Polymerization of Nonporous Polymer Sphere

Styrene and divinylbenzene in which the polymerization inhibitor was removed with activate alumina were mixed at 20 mL and 10 mL, respectively, adding benzoyl peroxide (0.23 g). The monomer solution was inserted to the solution (200 mL) including polyvinyl alcohol (2.0 g) and the polymerization was started at 700 rpm and 353 K for 2 hours. After the polymerization, the obtained sphere was washed with pure water and acetone repeatedly to recovery it by filtration in vacuum. The sphere was sieved dependent on sizes. The surface of the sphere was observed by optical microscopy (Keyence VH-500). The specific surface area was determined by nitrogen gas adsorption (Belsorp-miniII-SP, BEL Japan Inc.).

Modification of Dextran on Polymer Sphere

Fifty mg of the obtained sphere was mixed with 0.2 U/mL of DSase solution (5 mL) dissolved in 10 mM acetate buffer (pH 5.5) for 1 hour to immobilized DSase. The size of the modified sphere was ranged from 100 to 200 μm and from 300 to 500 μm. After immobilizing DSase, DSase-immobilized sphere was washed with acetate buffer. The amount of immobilized DSase was determined from the remained activity of DSase in the solution after the immobilization. An activity of 1 U was defined as the amount of enzyme required to produce 1 μmol of fructose in 1 min. The amount of DSase immobilized was evaluated from the activity of DSase.

The as-prepared DSase-immobilized sphere was reacted with a sucrose solution at the concentration of 10 and 100 g/L (10 mM acetate buffer, pH 5.5, 10 mL)

for 72 h at 303 K to produce dextran from the active sites of the immobilized DSase. The concentration of fructose, a by-product of the enzymatic reaction, was determined by evaluating the amount of dextran produced using the Somogyi-Nelson method (Somogyi, 1952; Nelson, 1944). The dextran-modified sphere was observed by optical microscopy (KEYENCE VE-9800).

The DSase immobilized on the sphere was calculated from as follow,

The amount of immobilized DSase [U/g-sphere] = $(C_0\text{-}C)\ V\ /W$ (1)

Where C_0, C, V, and W were the initial concentration of DSase U/mL, the concentration of DSase after immobilization U/mL, volume of solution, and weight of sphere g, respectively.

Concanavalin A Adsorption to the Dextran-Modified Sphere

Con A were dissolved in 10 mM acetate buffer at pH 5.5. Individual protein solution (5 mL) was mixed with the non-modified polymer material and with the dextran-modified polymer for 4 h at 303 K. The solution was filtered to remove the polymer, and subsequently the concentration of Con A in the remaining solution was determined by UV/VIS (Hitachi 3100, Japan). The amount of adsorbed Con A was defined as follow based on the molecular weight of Con A (51 kDa);

The amount of Con A adsorbed [mmol/g-sphere] = (the amount of protein adsorbed)] / (the amount of sphere) (2)

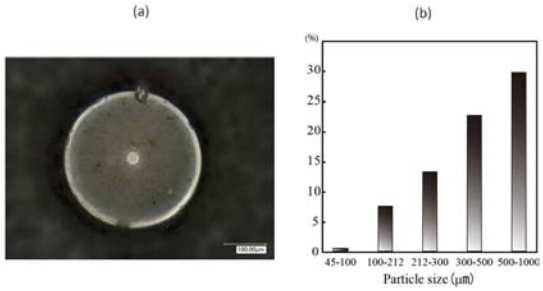

FIG. 2 OBTAINED HYDROPHOBIC PARTICLE (a) OPTICAL IMAGE AND (b) SIZE DISTRIBUTION OF PARTICLE

Results and Discussion

Modification of Sphere Surface by Dextran Generated from DSase's Reaction

The suspension polymerization was performed for the preparation of the hydrophobic sphere. Because the sphere was modified with dextran only on the surface, the sphere was expected to be polymerized as nonporous. The images by optical microscopy are shown in FIG. 2. The sphere had no pore and smooth morphology in the level of optical microscopy. The obtained sphere had the various ranges of the diameters from 10 to 500 m, as shown in FIG. 2 (b). In the following experimental, the sphere in the diameter of 100-200 μm and 300-500 μm was used for the modification with dextran. The specific surface area of the obtained sphere was less than 0.1 m^2/g, demonstrating that the sphere had nonporous structure.

DSase was immobilized on the surface of the hydrophobic sphere and sucrose was reacted with the immobilized DSase for the modification with dextran. Here, two spheres with the different sizes were immersed in DSase solution to immobilize DSase via hydrophobic interaction. Due to the nonporous structure of the sphere, DSase would be immobilized only on the surface. The primary structure of DSase has been already known. The hydrophobic amino acid in DSase was suggested to be interacted with the hydrophobic surface. After DSase immobilization, DSase-immobilized sphere was immersed to sucrose solution for the production of dextran on the surface.

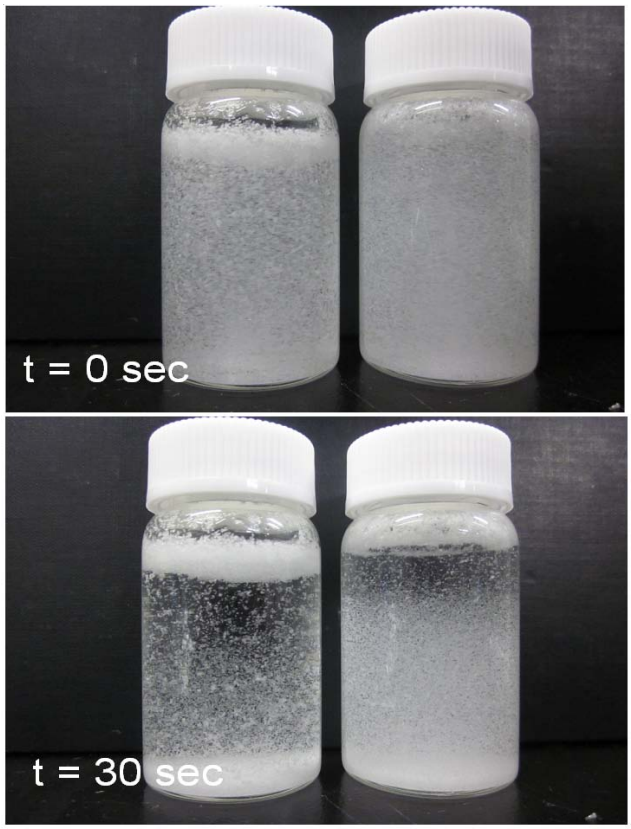

FIG. 3 IMAGES OF DISPERSION OF THE SPHERE. LEFT: UNMODIFIED AND RIGHT: DEXTRAN-PRODUCED SPHERE

The concentration of sucrose was set at 10 and 100 g/L. In processing the reaction with DSase, the viscosity of the solution was increased, indicating that the produced dextran on the sphere surface was entangled with each other in the solution. The images of the dextran-produced sphere and unmodified sphere dispersed in water are shown in FIG. 3. The precipitation speed of dextran-produced sphere was lower than that of unmodified sphere. Dextran has the high viscosity by the hydroxyl group and their entanglements. Modified spheres interacted with each other or with water to lower the precipitation speed in solution. During the use for the following adsorption, no leakage of dextran was observed.

The coverage with dextran on the different two spheres was summarized in TABLE 1. The amount of immobilized enzyme was set at constant. From the amount of immobilized DSase per the surface area, the number of immobilized DSase per the surface area of the sphere was possibly calculated as follows,

TABLE 1 PROPERTIES OF DEXTRAN-PRODUCED SPHERES

	conc. of sucrose [g/L]	100	10
sphere size [mm]	amount of DSase immobilized [U/g-sphere]	3.27	3.24
100 - 200	the number of DSase immobilized per the surface area of the sphere [number/m^2-sphere]	8.93×10^{15}	8.84×10^{15}
	the amount of dextran produced [mg/g-sphere]	13.3	4.28
300 - 500	amount of DSase immobilized [U/g-sphere]	3.26	3.2
	the number of DSase immobilized per the surface area of the sphere [number/m^2-sphere]	2.37×10^{16}	2.32×10^{16}
	the amount of dextran produced [mg/g-sphere]	13.5	4.36

$$Q\ [\text{number/m}^2]=(q_{DSase}\ N_A\ d_{sphere}\ V_{sphere})\ /\ (d_{DSase}\ M_{DSase}\ S_{sphere}) \quad (3)$$

where q_{DSase}, N_A, d_{sphere}, V_{sphere}, S_{sphere}, d_{DSase}, and M_{DSase} were the amount of DSase immobilized U/g-sphere, Avogadro number number/mol, the density of sphere g-sphere/m^3, the volume of the sphere m^3, the surface area of the sphere m^2, activity of DSase per powder of protein in the initial sample U/g, and molecular weight of DSase g/mol, respectively. The density of the sphere used was 1.05×10^6. It was assumed that all the contained protein was DSase. The amount of immobilized DSase per sphere surface area was different and the smaller the size was, the larger the number of immobilized DSase was. Based on the assumption that all the immobilized DSase was active, the molecular weight of the produced dextran was easily calculated as follows,

$$\text{Molecular weight of dextran} = [(q_{\text{produced dextran}}\ d_{DSase}\ M_{DSase})\ 10^{-3}/\ (N_A\ q_{DSase})]/162 \quad (4)$$

where $q_{\text{produced dextran}}$ was the amount of dextran produced mg/g-sphere. The value of 162 was molecular weight of glucose monomer in dextran. The produced dextran was calculated to be more than 10,000 kDa. The size of dextran at the molecular weight of 2,000 kDa is 20 nm as a hydrodynamic radius (Ioan, et al., 2000). Seto, et al. reported that in a batch mode more than 2,000 kDa of dextran was produced by DSase, determined by gel permeation chromatography (Seto, et al., 2008). On the hydrophobic surface, the giant macromolecule of dextran was possibly produced from the immobilized DSase. It was already found that by the immobilization in a laminar flow in circular tube, the same giant dextran of 20 μm was produced on the surface of the tube in our precious study (Miyagawa, et al., 2012).

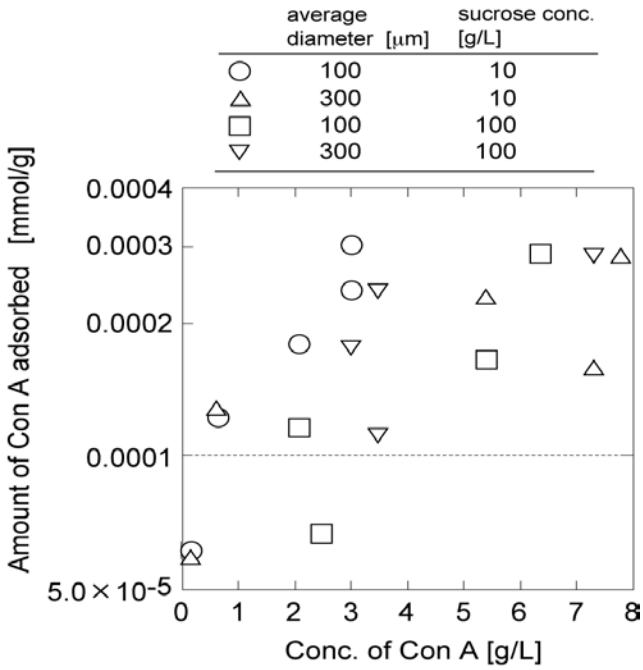

FIG. 4 ADSORPTION OF CON A TO THE FOUR KINDS OF DEXTRAN-PRODUCED SPHERE

Con A Adsorption with Dextran on Hydrophobic Sphere

Dextran with various densities and lengths was produced on the sphere surface by changing the size of sphere and substrate concentration of sucrose. To understand the adsorption characteristics of the produced dextran, Con A was adsorbed to the produced dextran in a batch mode. The relationship between the concentration of Con A at equilibrium and the amount of Con A adsorbed is shown in FIG. 4. During Con A adsorption, no leakage of dextran from the sphere was determined. Here, unmodified sphere, starting sphere, did adsorb Con A just a little bit via nonspecific hydrophobic interaction. With increasing in the concentration of Con A at equilibrium, the amount of Con A adsorbed was increased. Con A can

be captured selectively to the glucose residue of dextran. The produced dextran had the random structure, having the high volume of steric hinderance, resulting that Con A adsorption was strongly related to the length and density of produced dextran.

Con A adsorption to the produced dextran was analyzed using the following Freundlich curve to determine the Freundlich constant and saturated capacity.

$$F = KC_F^{1/n} \quad (5)$$

TABLE 2 ANALYSIS OF CON A ADSORPTION TO DEXTRAN-PRODUCED SPHERE USING FREUNDLICH CURVE

sphere size [μm]	conc. of sucrose [g/L]	100	10
100 - 200	Freundlich constant K	1.12×10^{-4}	1.50×10^{-4}
	adsorption index n	2.94	2.16
300 - 500	Freundlich constant K	7.50×10^{-11}	1.23×10^{-4}
	adsorption index n	1.06	3.27

where K, C_F, and n were Freundlich constant, the Con A concentration after adsorption, and adsorption index, respectively. Here, the nonspecific interaction between Con A and the sphere was neglected. Freundlich constant was directly related to the strength of adsorption. The Freundlich constant and adsorption index in the adsorption of Con A are summarized in TABLE 2. The sphere at the size of 300 – 500 μm in reacting with sucrose of 100 g/L has the lowest Freundlich constant. Along with the data in TABLE 1, the above-mentioned sphere had the higher number of DSase immobilized per area and amount of immobilized DSase. These data indicated that due to the steric hinderance with dextran from its random structure, the reduction of the affinity to Con A occurred. Generally, with increasing in the amount of produced dextran, the saturated capacity gradually increased due to the enhancement of the glucose as an adsorption site. The recognition site of Con A is the non-reducing site in the terminal of dextran, already found by Goldstein, et al (Goldstein, et al., 1965a, 1965b). At present, the density of non-reducing site in the terminal of branched dextran was not yet determined. As Con A was adsorbed to the produced dextran, the dextran would have the branched structure with the non-reducing end. For the polymer-brush-like structure on the surface, the excluded volume of the polymer should be considered for designing adsorbent.

The appropriate design of dextran in density and length is required for using the adsorption site. Using the characteristics of DSase, the immobilized density of DSase and the reaction condition with sucrose are directly related to the design concepts. The polymer design concept of this dextran formed on the surface is similar to the polymer brush with RAFT and ATRP methods. The modification of the surface with dextran in this study was applicable if the following two items are acceptable, 1) the immobilization of DSase on the surface, and 2) the active of DSase on the surface. This technique including the DSase immobilization and production of dextran on the hydrophobic surface enable us to use the surface modification of the medical, analytical, reaction, and separation fields.

Conclusions

The hydrophobic sphere in micrometer was covered with dextran produced via dextransucrase's reaction. Dextransucrase, a kind of transferase, was immobilized on the nonporous sphere to react with sucrose, producing dextran from the immobilized dextransucrase. The hydrophobic sphere was polymerized. In changing the sphere size and substrate concentration, sucrose, the produced dextran was changed in length and density. After the adsorption of concanavalin A with the produced dextran, the length and density of dextran should be designed for the high performance of an adsorbent.

Notations

C	concentration of DSase after immobilization
C_F	Con A concentration after adsorption
C_0	initial concentration of DSase
d_{DSase}	activity of DSase per powder of protein in the initial sample
D_{sphere}	density of sphere
K	Freundlich constant
M_{DSase}	molecular weight of DSase
N_A	Avogadro number
Q	number of DSase immobilized per the surface area of the sphere
q_{DSase}	amount of DSase immobilized
$q_{produced\ dextran}$	amount of dextran produced
S_{sphere}	surface area of the sphere
V	volume of solution
V_{sphere}	volume of the sphere
W	weight of sphere
n	adsorption index

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